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Pyrrolidinoindoline alkaloids from *Margaritopsis cymuligera* (Muell. Arg.) C.M. Taylor (Rubiaceae)

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1. Subject and source

Margaritopsis cymuligera (Muell. Arg.) C.M. Taylor (tribe Psychotrieae, family Rubiaceae) is a shrub which occurs in Southeastern and South Brazil, and Northeastern Paraguay (Bacigalupo et al., 2008; Taylor and Zappi, 2010). Plant material (leaves) was collected in December 2008, in Dom Pedro de Alcântara (S 29°22.851′; W 049°50.025′), Rio Grande do Sul, Brazil, and identified by Dr. Sérgio Bordignon of the Centro Universitário La Salle. A voucher specimen is deposited at the herbarium of Universidade Federal do Rio Grande do Sul (UFRGS) (ICN 189954).

2. Previous work

Based on investigations on the tribe Psychotrieae (Rubiaceae) many species of *Psychotria* were transferred to *Margaritopsis* genus (Andersson, 2001, 2002; Taylor, 2005). The species *Psychotria birotula* was reclassified as *M. cymuligera* (Muell. Arg.) C.M. Taylor. To our knowledge, no phytochemical investigation of this species has been reported in the literature.

Among the species of *Psychotria* that were included by Andersson (2001) as *Margaritopsis*, *Psychotria oleoides* (from New Caledonia) is the unique species investigated chemically and showed pyrrolidinoindoline alkaloids as major constituents (Libot et al., 1987; Guéritte-Voegelein et al., 1992; Jannic et al., 1999).

Pyrrolidinoindoline alkaloids are an important metabolic group found in *Psychotria* and several of them were already isolated from this genus, such as isopsychotridine C, psychotridine, quadrigemines A and C, from *Psychotria forsteriana* (Roth et al., 1986); chimonanthine, hodgkinsine, quadrigemine, psychotrimine and psychopentamine from *Psychotria rostrata* (Takayama et al., 2004; Lajis et al., 1993); chimonanthine, hodgkinsine and quadrigemine C from *Psychotria colorata* (Verotta

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et al., 1998); *meso*-chimonanthine, *Nb*-desmethyl-*meso*-chimonanthine and hodgkinsine from *Psychotria lyciiflora* (Jannic et al., 1999); hodgkinsine, quadrigemine C, isopsychotridine B, psychotridine, quadrigemine I, oleoidine, and caledonine from *P. oleoides* (Jannic et al., 1999).

3. Present study

Dried and ground leaves (215 g) were extracted at room temperature with MeOH and the solvent evaporated under vacuum. The crude extract was dissolved in MeOH:water 1:1 and submitted to a partition into hexane and ethyl acetate. The reminiscent methanol-aqueous fraction was subjected to an acid–base extraction, yielding 0.94 g of alkaloid extract. Part of this extract was purified by HPLC (Thermo, Mod. Chrom-Quest chromatograph) with a DAD detector, using a Microsorb 100-5 RP-18 column (250 × 10.0 mm) and MeOH/H_2O/Et_3N (75:25:0.1) to MeOH/H_2O/Et_3N (85:25:0.1) as solvent, resulting in a fraction (15 mg) whose ^1H and ^{13}C NMR spectra indicated the presence of a mixture of pyrrolidinoindoline alkaloids (Jannic et al., 1999; Verotta et al., 1998): ^1H NMR δ $CDCl_3$ 2.01 (s), 2.40 (s), 2.07 (m), 2.10 (m), 2.54 (m), 2.81 (m), 2.86 (m), 2.90 (m), 4.80 (s), 6.62 (d, $J = 7.8$), 6.63 (d, $J = 7.8$), 6.64 (t, $J = 7.2$), 6.75 (d, $J = 7.2$), 6.92 (d, $J = 7.2$), 7.00 (t, $J = 7.2$), 7.17 (t, $J = 7.2$). ^{13}C NMR: δ $CDCl_3$ 35.8, 36.2, 38.7, 39.5, 52.6, 52.9, 61.1, 61.7, 86.2, 86.8, 109.0, 109.4, 119.5, 123.6, 124.4, 125.9, 126.0, 129.1, 133.2, 133.4 149.6, 152.1. This fraction was acetylated with Ac_2O in pyridine, at room temperature, for 72 h and the resulting product subjected to GC-MS analysis. From this analysis was possible identify two principal compounds with molecular ion peaks at *m/z* 644 and *m/z* 858 in the EI-MS spectra (70 eV). The compound with molecular peak at *m/z* 644 was identified as N_a,N_a',N_a''-triacetyl hodgkinsine: *m/z* (rel. int. %) 644 (M^{+}•, 10), 601 (10) [M^{+}• − Ac], 558 (10), 515 (10), 415 (70) [M^{+}• − (171 + Ac + Me)], 285 (50), 284 (60), 271 (85) and 100 (100). The peak at *m/z* 858 [M^{+}•] was correlated to a tetra-acetylated quadrigemine type

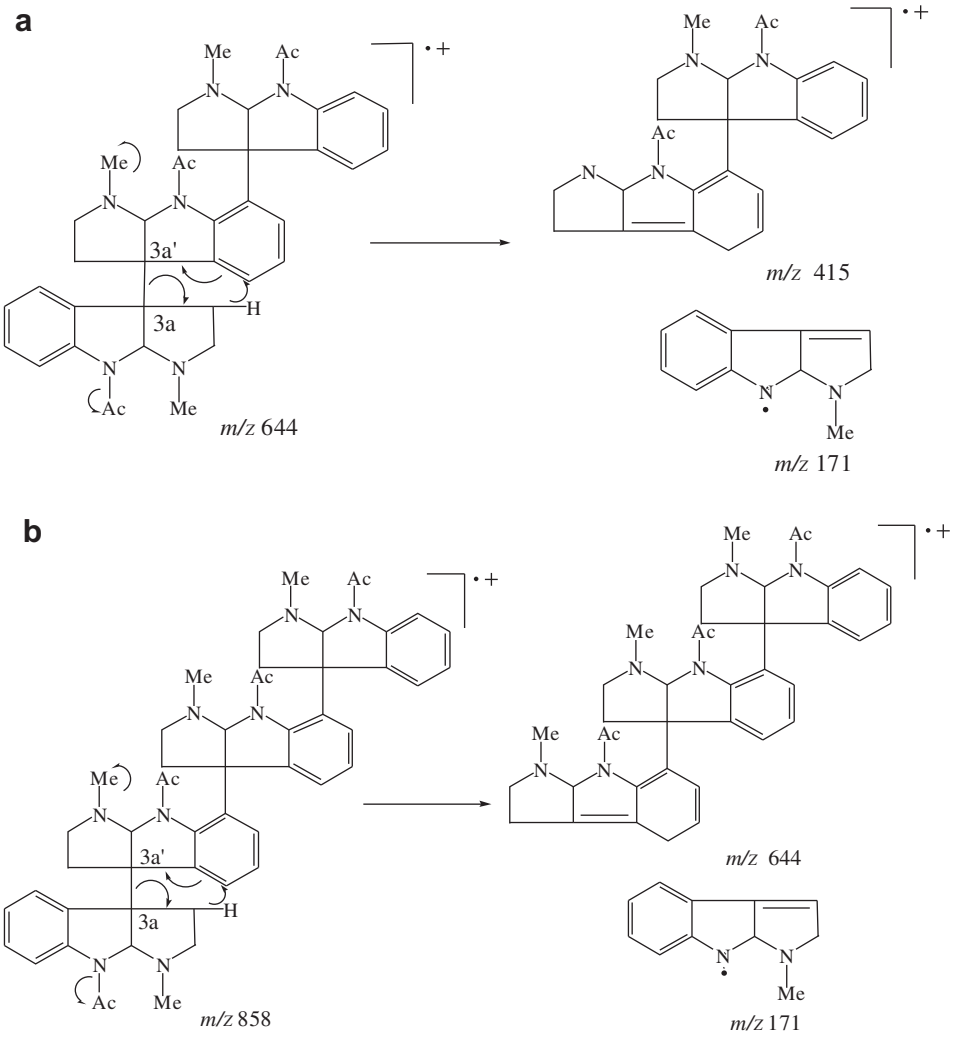

Fig. 1. Mechanisms proposed for the fragmentations of isolated pyrrolidinoindoline alkaloids: a) *m/z* 644; b) *m/z* 858.

alkaloid (N_a,N_a',N_a'',N'''-tetraacetyl quadrigemine): *m/z* (rel. int.%) 858 ($M^{+\bullet}$, 1), 815 (1) [$M^{+\bullet}$ − Ac], 644 (15) [$M^{+\bullet} - (171 + Ac)$], 601 (70) [$M^{+\bullet} - (171 + 2Ac)$], 528 (40) [$M^{+\bullet} - (171 + 3Ac + 2Me)$], 515 (65), 386 (30), 271 (45), 100 (100). The presence of the fragments originated by loss of 171 u plus acetyl and methyl groups from the molecular peak [$M^{+\bullet}$], evidenced the terminal *Nb*-methyltryptamine unit linked by a C-3a–C-3a′ to a group of two or three units (Fig. 1). According Jannic et al. (1999), the fragmentation of pyrrolidinoindoline alkaloids having a terminal *Nb*-methyltryptamine unit linked by a C-3a–C-3a′ can occurs by three different pathways with loss of 172, 173 or 174 u from the $[M + H]^+$ ions. This pattern of fragmentation was reported for unsymmetrical quadrigemine-type alkaloids like quadrigemines B and I, in contrast with the observed for the symmetrical quadrigemines A and C, which presents an intense peak at *m/z* 344, corresponding to two pyrrolidinoindoline units (Guéritte-Voegelein et al., 1992; Jannic et al., 1999; Libot et al., 1987; Verotta et al., 1998).

4. Chemotaxonomic significance

The pantropical genus *Margaritopsis* C. Wright includes more than 50 ssp., with 27 neotropical species of shrubs, and at least 25 paleotropical species. The genus name *Margaritopsis* was originality restricted to three species found in dry forests in Cuba and Hispaniola (Taylor, 2005). Based on molecular and cladistic investigations on the tribe Psychotrieae (Rubiaceae), Andersson (2001, 2002) concluded that *Margaritopsis* should be expanded to include the Pacific genus *Readea* Gillespie, the African genus *Chazaliella* E.M.A. Petit & Verdc., the widespread neotropical species *Psychotria microdon* (DC.) Urb., and the South American species that have been included in *Psychotria* sect. *Chytropsia* (Bremek.) Steyerm. The investigations discriminated a monophyletic, pantropical group of at least 50 spp., for which *Margaritopsis* proved to be the oldest generic name available (Andersson, 2001; Taylor, 2005). Most of these species are found in Africa (ca. 24), the neotropics (ca. 10) and New Caledonia and Fiji (ca. 12), and a few species in South East Asia, Indomalayan area and New Guinea. Also, three new combinations are made: *Margaritopsis membranacea* (Gillespie) L. Andersson, *Margaritopsis astrellantha* (Wernh.) L. Andersson, and *Margaritopsis abrupta* (Hiern) L. Andersson.

A study of the neotropical members of *Margaritopsis* group was further made by Taylor (2005), which enumerated 27 spp., ranging from southern Mexico to Bolivia and Paraguay and in the Antilles. All were formerly classed in *Psychotria*, except for the three spp. original from Antillean and one sp. from South America. The list of names for neotropical *Margaritopsis* based on the review by Taylor (2005) includes the species *M. cymuligera* (Muell. Arg.) C.M. Taylor, which is the accepted name for *P. birotula* L.B. Sm. & Downs.

Due to the relative complex taxonomy of the tribe Psychotrieae and the fact that only one species of *Margaritopsis* genus (formerly *P. oleoides*) was chemically evaluated, investigations on alkaloid contents in *M. cymuligera* may contribute to further taxonomic studies. The pyrrolidinoindoline alkaloids hodgkinsine and quadrigemines C and I were reported to occur in *P. oleoides* (Jannic et al., 1999), a species recently reclassified as *Margaritopsis*. The presence of pyrrolidinoindoline alkaloids in Margaritopsis cymuligera may contribute to further taxonomic studies of Margaritopsis genus and of tribe Psychotrieae.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bse.2012.07.009.

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